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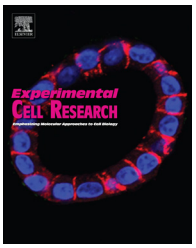

Research Article

A fat option for the pig: Hepatocytic differentiated mesenchymal stem cells for translational research

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ABSTRACT

Study background: Extended liver resection is the only curative treatment option of liver cancer. Yet, the residual liver may not accomplish the high metabolic and regenerative capacity needed, which frequently leads to acute liver failure. Because of their anti-inflammatory and -apoptotic as well as pro-proliferative features, mesenchymal stem cells differentiated into hepatocyte-like cells might provide functional and regenerative compensation. Clinical translation of basic research requires pre-clinical approval in large animals. Therefore, we characterized porcine mesenchymal stem cells (MSC) from adipose tissue and bone marrow and their hepatocyte differentiation potential for future assessment of functional liver support after surgical intervention in the pig model.

Methods: Mesenchymal surface antigens and multi-lineage differentiation potential of porcine MSC isolated by collagenase digestion either from bone marrow or adipose tissue (subcutaneous/visceral) were assessed by flow cytometry. Morphology and functional properties (urea-, glycogen synthesis and cytochrome P450 activity) were determined during culture under differentiation conditions and compared with primary porcine hepatocytes.

Results: MSC from porcine adipose tissue and from bone marrow express the typical mesenchymal markers CD44, CD29, CD90 and CD105 but not haematopoietic markers. MSC from both sources displayed differentiation into the osteogenic as well as adipogenic lineage. After hepatocyte differentiation, expression of CD105 decreased significantly and cells adopted the typical polygonal morphology of hepatocytes. Glycogen storage was comparable in adipose tissue- and bone marrow-derived cells. Urea synthesis was about 35% lower in visceral than in subcutaneous adipose tissue-derived MSC. Cytochrome P450 activity increased significantly during differentiation and was twice as high in hepatocyte-like cells generated from bone marrow as from adipose tissue.

Abbreviations: pBM-MSC, porcine bone marrow-derived mesenchymal stem cells; pATsub-MSC, porcine subcutaneous adipose tissue-derived mesenchymal stem cells; pATvis-MSC, porcine visceral adipose tissue-derived mesenchymal stem cell

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Conclusion: The hepatocyte differentiation of porcine adipose tissue-derived MSC was shown for the first time yielding hepatocyte-like cells with specific functions similar in bone marrow and subcutaneous adipose tissue-derived MSC. That makes them good pre-clinical candidates for supportive approaches after liver resection in the pig.

Introduction

Currently, liver resection represents the only option of treatment for most patients suffering from primary or secondary liver tumors. Extended resections of up to 70% of the liver mass necessitate the residual organ to take over the metabolic capacity until regeneration is completed [1]. Yet, trauma, inflammation and ample tissue remodeling after liver surgery often result in acute failure namely post-hepatectomy liver failure (PHLF) with fatal consequences for the patient. Liver transplantation is a contraindication because of the high risk of recurrence. Hepatocyte transplantation instead of whole liver transplantation is a feasible alternative. It is based on the assumption that healthy donor-derived hepatocytes transplanted into the host liver engraft and functionally repopulate and reconstitute the recipient organ in the long range. 1–5% of liver repopulation by donor hepatocytes may suffice to correct for a monogenetic metabolic defect in the liver [2,3]. Patients with Crigler–Najjar syndrome [4] or glycogen storage disease type 1a [5] improved after hepatocyte transplantation allowing to bridge to liver transplantation. Thus, hepatocyte transplantation has reached proof-of-concept in numerous clinical trials [6–8]. However, the scarcity of donor livers for whole organ transplantation as well as for hepatocyte isolation demands for novel therapeutic approaches. Alternative cell sources like mesenchymal stem cells (MSC) gained attention first in pre-clinical but now in clinical settings as well to generate stem cell-based hepatocytes [7]. Both in rodents [9–11] and humans [12–18] it was unequivocally shown that MSC from bone marrow or adipose tissue displayed hepatocyte differentiation potential in vitro.

These pre-clinical experiences hold great promise for the utilization of hepatocyte pre-differentiated MSC in the therapy of liver diseases. Yet, anatomical, physiological and biochemical differences between rodents and humans do not allow for direct translation into clinical trials. Particularly in the field of surgery it must therefore be the goal to develop a supportive system in the case of extended liver resection in a large animal model, preferably in pigs, to gain basic essential insight into the outcome, therapeutic benefits and safety of cell therapeutic approaches. Indeed, first evidence of hepatocyte differentiation of porcine bone marrow-derived MSC was provided by Groth and colleagues showing onset of hepatocyte-specific gene expression on the mRNA level after culture of the cells under specified differentiation conditions [19]. Their functional properties after hepatocyte differentiation have been investigated recently [20].

The present study describes for the first time the isolation of MSC from visceral and subcutaneous adipose tissue and compares their functional hepatocyte properties with those in bone marrow-derived MSC. Hepatocyte differentiation conditions promoted hepatocyte-specific functions like urea and glycogen synthesis as well as cytochrome P450 enzyme activity comparable to cultured primary porcine hepatocytes.

Material and methods

Isolation of porcine MSC

All animal experiments were approved by the regulatory authorities of the German Federal State Saxonia, and the animal protection commissary of the University Leipzig, Germany. pBM-MSC and pAT-MSC were isolated from adult female landrace pigs (30–40 kg body weight). To harvest the *Substantia spongiosa* for isolation of pBM-MSC ($n=3$) the *Os femoris* was exposed and opened. pATvis-MSC ($n=3$) and pATsub-MSC ($n=3$) were isolated from collected visceral or subcutaneous adipose tissue, resp. All tissue samples were digested with collagenase (NB4G, Serva, Heidelberg, Germany) at 37 °C for at least 20 min. Subsequently, cells were separated by density gradient centrifugation (BIOCOLL SEPARATING SOLUTION, Biochrom, Berlin, Germany). The mononuclear cell fraction was then collected, seeded onto plastic cell culture dishes and grown to 90% confluence in stem cell maintenance medium (EM6F) as described previously [21].

FACS-analysis

Flow cytometry data were acquired by the Fluorescence Technology Core Unit IZKF in Leipzig (Germany) using the BD LSRII and BD FACS Diva v.6.1.3 software as described previously [22]. The following marker panel was evaluated before and after hepatocyte differentiation: CD45 (MA1-81121, Pierce, Bonn, Germany), CD14 (ABIN118506, antikoerper online), CD44 (ab19622, Abcam, Cambridge, UK), CD90 (555595, BD, Heidelberg, Germany), CD105 (ab53318, Abcam, Cambridge, UK) and CD29 (561496, BD, Heidelberg, Germany).

Hepatocyte differentiation

Hepatocyte differentiation of MSC was initiated by adding growth factors as described by Stock et al. [23] and continued for another 21 days. Briefly, cells were grown to 90% confluence, then treated with 20 μM of 5′azacytidine for 24 h. Cells were washed and the growth medium changed to hepatocyte differentiation medium supplemented with 2% fetal calf serum, HGF and EGF as described in detail in [23]. Cells were cultured for the times indicated and the medium changed every four days. Functional assays for CYP1A1 and CYP2B1 activity and urea synthesis were performed at day 0, 7, 14 and 21 of differentiation.

Osteogenic and adipogenic differentiation

For adipogenic and osteogenic differentiation, MSC were cultivated until 90% confluence. Differentiation was initiated by adding 25 μM azacytidine (Sigma-Aldrich, Steinheim, Germany) and culture continued for 24 h. Thereafter, specified differentiation